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Foliar organization in twenty species of the Ranunculaceae

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FOLIAR ORGANIZATION IN TWENTY SPECIES OF THE RANUNCULACEAE

by

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INTRODUCTION

The early studies of foliar organization were involved primarily with gross aspects of leaf anatomy. The first general description of the dorsiventral dicotyledonous leaf was given by Brogniart and Treviranus in 1830 (in Haberlandt, 1914). Nordhausen (1903, in Sinnott, 1960) did the first extensive anatomical study on sun and shade leaves. These early investigations revealed that variations in internal structure exist between the leaves of different species and between leaves from different parts of the shoot system of the same plant. Foliar studies became ecologically and physiologically oriented in an attempt to seek and explain the causal factors for these observed and reported differences. There is probably no measurable environmental factor, the influence or potential influence of which might be thought to cause modifications in leaf form and structure, which has not been the subject of some research.

Water relations of land plants are recognized as being physiologically critical (Kramer, 1949). The leaf, the site of transpirational water loss, has been the primary organ studied in investigations on the effects of the availability of water to the plant. Coulter, et al. (1931) reported that the chief determining factors in leaf size and proportions were those which control water supply. A high

transpiration rate, whether caused by dry soil, high salt content, low oxygen pressure, or high temperature, was the dominating factor determining the small size and great thickness of xeric leaves. Weaver and Clements (1938) reported an apparent correlation between the extent of lobing in leaves and the rate of water loss from the plant. Daubenmire (1947) cited smaller leaf size and internal structural modifications of the leaf as being induced in plants grown under an unfavorable water balance. Uziak (1952) compared the density of veins in leaves of plants of different habitats. She concluded that the differences in density observed were adaptations to development under different ecological conditions particularly as concerned with the evaporation of water in the environment.

The work of Nordhausen (1903, in Sinnott, 1960) led to numerous investigations on the effect of insolation on leaf structure. This remains a popular area of study. The effects of insolation on gross external form were studied by Anderson (1955). He found sun leaves to be thicker, darker in color, with more prominent veining and marginal serrations than shade leaves. Talbert and Holch (1957) observed more prominent lobing in sun leaves of Acer spp. as compared with shade leaves. Duncan (1959), initially investigating the differences in gross morphology in sun and shade leaves of single trees of Liquidambar styraciflua L., reported greater variations

between leaves of trees of this species growing in different parts of its natural range than he found between sun and shade leaves of any one tree. Minor morphological differences between ecotypes, perhaps expressive of minor genetic differences, are currently receiving the attention of ecologists and geneticists.

Modifications in the internal organization of leaves due to varying amounts of insolation have been described by Esau (1953) and reported by Wylie (1951) and Thompson (1943). On the basis of these studies, extensive development of the palisade tissue was associated with sun leaves while spongy mesophyll development was associated with leaves maturing at reduced light intensities. Isanogle (1944) studied the effects of controlled shading upon the development of leaf structure. She observed that internal differences between sun and shade leaves became more apparent as the leaves approached maturity. Cormack and Gorham (1953) observed the extremes of insolation effects on leaf structure. Their results indicated that leaves possess great plasticity and a capacity for structural modification in response to a severe change in insolation. Experimental evidence from this study refuted Nordhausen (1903, in Sinnott, 1960) who stated that shade buds produced shade leaves and sun buds produced sun leaves regardless of the illumination during development. Cormack (1955), in another study on insolation and leaf form, found that, unlike the

condition which develops in the normal sun-shade relationship between leaves of the same plant, extreme shade leaves were smaller in overall size than sun leaves. In a comparative anatomical study of normal shade and extreme shade leaves of Convallaria, Cormack (1962) found that the mesophyll cells of the extreme shade leaves failed to enlarge.

Investigations have also been made on the effect of insolation on specific structural and functional manifestations. Pieters (1960) established a linear correlation between the maximum rate of photosynthesis and leaf thickness; sun leaves of Acer pseudoplatanus L. were found to be the thickest and to have the highest photosynthetic rate. In reference to palisade tissue, Pick (1881, in Watson, 1942) and Stahl (in Sachs, 1887) reported that palisade formation depended on intense light. Sachs (1887), Pfeffer (1903), Jost (1907), Haberlandt (1914), and Watson (1942) maintained that the effects of insolation on palisade development were only quantitative. According to Esau (1953), the establishment of palisade in the developing lamina is a genetic trait and is, therefore, independent of light.

The effects of temperature on leaf morphology have been the subject of fewer investigations than have been other environmental factors. Temperature apparently is more indirectly operative in modifying leaf morphology, affecting metabolic activity including the rate of leaf production at the growing tip which, in turn, influences

the lobing of leaves and other features of general morphology (Sinnott, 1960). Fisher (1954) worked with a trifoliate species of Ranunculus which has undivided juvenile leaves. He induced complete reversion of later formed leaves to the undivided juvenile condition by subjecting the study plants to daytime temperatures of 20 degrees C. and night temperatures of 15 degrees C. At lower temperatures, the adult trifoliate leaf form persisted.

The impact of special habitat types on leaf form has been studied as well as isolated environmental factors. Müller-Stoll (1947, in Sinnott, 1960) and Philpott (1956) studied morphological modifications in leaves of plants associated with bog environments. Shields (1949) observed leaf xeromorphy in dicotyledons from a gypsum sand deposit. Gregory (in Milthorpe, 1956) regarded any environmental complex as important insofar as it affects the general nutritive conditions under which a plant grows.

Morphological modifications of the internal structure of the leaf as associated with physiological factors are more difficult to demonstrate than are those associated with environmental factors. The development of the palisade portion of the mesophyll as a function of transpirational activity was investigated (as in Watson, 1942) by Eberdt (1887), Wagner (1892), and Lothelier (1893). Hanson (1917) and Turrell (1936) regarded extensive palisade development as causal to high

transpirational rates. According to the latter, it is the exposed internal surface area and not the volume of the intercellular space which influences transpiration rates in leaves. Palisade, therefore, is not a barrier to transpiration, but rather its development to any extent increases internal surface area which favors transpiration.

As an organ of translocation, the association between the mesophyll organization of leaves and venation was studied by Mounts (1932), Philpott (1947), and Wylie (1946, 1951). Ontogenetic studies of venation have been made by Foster (1950, 1952) and Pray (1955, 1959). Functionally associated with the vascular tissue and the cells of the mesophyll region, the bundle sheath was studied by Wylie (1938) and Armacost (1944). Plymale and Wylie (1944) reported that bundle sheath extensions greatly increase the conductive capacity of minor veins by increasing the contact area of the vein with the cells of the mesophyll.

The solution to the problem as to why leaves vary has been sought in general morphological and anatomical studies. Zalenski (in Shields, 1949) observed that in any one shoot, the anatomical structure of the individual leaf is a function of its distance from the root system. Clowes (1956) generalized that the ultimate size of the expanded cells in a given leaf is determined by its position on the shoot. Maksymowych (1959) reported that anatomical differences in

any one leaf are due to differential rates of maturation of tissues. In vascular and nonvascular tissues, maturation proceeds basipetally.

While the foregoing emphasizes work concerned with the differences in leaf structure, Philpott (1953) suggested that similarities observed in foliar organization of plants dissimilar in gross morphology and in their habitat preferences might be of physiologic significance, especially if such similarities were analogous.

From this review of investigations, which is not presumed to be complete, a judgment can be made on the amount of work already done on foliar organization. However, with the exception of economically important species, such as Nicotiana spp., the leaves of herbaceous plants have been the object of much less research than those of woody species. Wylie (1952) stated that the foliar organization of herbaceous plants needed attention. In the conclusion of his work on the shoot apex of Clematis, Tepfer (1960) suggested that further studies on mature leaves of the genera Clematis, Ranunculus, and Aquilegia would be helpful.

There have been no comprehensive studies made on the foliar organization of an herbaceous plant family. There is a lack of information in the literature on the most general aspects of herbaceous leaf anatomy. References to the "typical" mesophytic leaf seem inconsistent in view of the quantity of work on leaf variation. Does

dorsiventrality, thus far a consistently reported feature of mesophytic dicotyledonous leaves, make them "typical"? Is there a similarity of internal structure in morphologically similar leaves of taxonomically related species? Is it possible to observe internal similarities in leaves of taxonomically related species growing under similar environmental conditions? Can certain aspects of foliar organization be associated with habitat type? Do principles of foliar organization observed under special environmental circumstances apply generally to internal leaf structure? The purpose of the current study is to initiate answers to these questions by studying internal leaf structure as manifested in an herbaceous dicotyledonous family, the Ranunculaceae.

Although confined to species of one plant family a study need not be restrictive. In the Ranunculaceae there is a variety of leaf forms represented. According to Gray (1950) some of the genera in this family and, occasionally, species within a genus, are confined to a certain general type of habitat; i.e., species which occur only in wet areas, species of open meadows, species of dry woods. While representing different habitats, the species in this study, with one exception, are not indicators of special environments and, with the exception of one cultivated species, are naturally occurring in northeastern Ohio. The native species under study were sampled from

their respective typical environments, insofar as these were outlined in Gray (1950), from undisturbed sites within conservation districts of the Cleveland Metropolitan Park System.

In addition to contributing information on herbaceous leaf anatomy, the results of this study will be used to evaluate the application of reported principles of foliar organization obtained primarily from studies on the leaves of woody plants and plants of special environments.

MATERIALS AND METHODS

Eleven of the twenty genera indigenous to the United States listed by Lawrence (1951) as members of the Ranunculaceae were used in this study. In selecting the species for study, the following criteria were observed: species which would have mature but not senescent foliage at the time the collections were made; species of common occurrence so that samples from a number of individuals occurring at a particular collection site could be obtained rather than samples from one or two isolated plants or from several collection sites; species taxonomically familiar to the collector; species with a known preference for a certain general type of habitat or confined, in the northeastern Ohio area, to a particular environmental locale. In addition to the nineteen native species selected according to these criteria, Paeonia albiflora was included as a representative of a locally cultivated variety of a tropical genus of the Ranunculaceae.

The species under consideration in this study are listed according to the general type of habitat in which they were found to occur and were collected in the northeastern Ohio area:

Open meadow: Ranunculus acris L.
R. acris var. latisectus G. Beck

Shale slope, sun:
Delphinium tricorne Michx.

Well drained, semi-open woodland:
Anemone cylindrica Gray
A. virginiana L.
Aquilegia canadensis L.
Ranunculus hispidus Michx.
Hepatica americana (DC.) Ker.

Moist woods: Ranunculus pensylvanicus L. F.
R. repens L.
Actaea pachypoda Ell.
Hepatica acutiloba DC.

Wet to swampy, sun:
Ranunculus ficaria L.

Wet to swampy, shade:
Ranunculus septentrionalis Poir.
Cimicifuga racemosa (L) Nutt.
Caltha palustris L.

Flood plain, sandy dry soil, shade:
Thalictrum polygamum Muhl.

Flood plain, sandy dry soil, sun:
Clematis verticillaris DC.

Wet edge of glacial bog, shade:
Coptis groenlandica (Oeder) Fern.

The leaf samples of Paeonia albiflora Pallas (hort.) were taken from plants growing in dry, clay soil in partial shade.

The leaf samples were collected during the first week of July. The midrib or main vein of a leaf was excluded; the samples were taken from the portion of the lamina between the midrib and lateral margin in an area equidistant from the tip and basal portion. In the study species which had compound leaves, a leaflet from an intermediate blade area was sampled. Samples from palmately dissected

simple leaves were taken from intermediate lobes. Seventeen of the species had cauline leaves. In these cases, the lowest and highest leaves on the shoot were omitted from the sampling in favor of leaves originating from the mid-shoot portion. Leaves of intermediate age were sampled from the three species which had a basal leaf arrangement.

Leaf samples measuring 4mm. x 5mm., the long dimension taken parallel to the long axis of the lamina, were excised with a scalpel from intact plants and placed immediately in glass vials containing a solution of 50% ethyl alcohol, propionic acid, and formalin in a ratio of 18:1:1. Twenty leaf samples of each species, representing at least fifteen individuals growing under the same environmental conditions, were collected. Within six hours after the leaf materials had been collected, they were taken to the laboratory and the air exhausted from them under a water suction pump, the apparatus used and the procedure followed being that described by Johansen (1940). The air exhaustion was carried out in two stages over a period of twenty-four hours in order to minimize the danger of plasmolysis. The tertiary butyl alcohol method of dehydration was followed according to Johansen (1940); the leaf portions were then subjected to a twenty-four hour infiltration process and subsequently embedded in Bioloidin paraffin.

Transverse sections of ten leaf samples of each species were prepared on a Spencer rotary microtome at a thickness of 10 microns. Haupt's adhesive, flooded with a 3% solution of formalin, was used in the mounting process. In that a warming table was not available, after the lengths of paraffin ribbon were transferred to the slides prepared with the adhesive and formalin flood, the slides were placed in an incubator which had a maintained temperature of 43 degrees C. where they remained for approximately fifteen minutes. This length of time was sufficient for the paraffin ribbons to flatten out without the formalin flood drying up. After the slides had been removed from the incubator and the excess formalin drained off, they were set aside for twenty-four hours to dry thoroughly before staining.

Approximately half of the remaining tissue samples were used in the preparation of paradermal sections at 12 microns. The remainder were used to prepare whole mounts of cleared leaf samples. In the clearing process the leaf samples were placed in a 5% solution of warm sodium hydroxide for from one to three hours, depending on the species, until the tissue portions were flexible. After three washings, the portions were bleached in a dilute solution of liquid chlorine bleach, washed again, and stained for approximately thirty minutes in a 0.5% solution of safranin 0 in 75% ethyl alcohol. After staining, the tissues were run through an 85%, 95%, and 100% alcohol dehydrating

series, placed in two changes of xylene, and mounted in Harleco Synthetic Resin. It was necessary to allow portions of thicker leaves to remain in the final dehydrating alcohol overnight in order to eliminate clouding when they were placed in xylene. All of the above described processes were carried out in small glass vials. Solutions were changed by pipetting rather than by decanting when it was found that the latter method damaged the tissues which were made fragile by treatment in sodium hydroxide.

Transverse and paradermal sections were stained with safranin 0 and fast green according to the schedule of Johansen (1940); the solutions were prepared according to Gray (1964).

In the study of the transverse sections, 50 measurements, 5 from each of the 10 leaf samples of each species collected, were made with the aid of a calibrated ocular micrometer at 440X. These measurements included the following: total blade thickness; thickness of the upper epidermis including the cuticle, if present; thickness of the palisade mesophyll; thickness of the spongy mesophyll; thickness of the lower epidermis. The measurements were recorded and averaged for each tissue sample. The 50 measurements obtained for each category mentioned above were averaged in order to establish mean values for the species. A record of individual sample variability was thus obtained along with average values.

The relative percentage of each blade tissue component, i.e., the thickness of the tissue component/blade thickness, was calculated for each species on the basis of the mean values derived, and for the thinnest and thickest samples. The tissue ratio for each sample and for each species was calculated by making use of the measurement data. The tissue ratio is the quotient derived from dividing the combined volumes of upper and lower epidermal tissues and the spongy mesophyll by the volume of palisade tissue.

The transverse sections were examined for the presence of bundle sheath extensions, parenchymatous cells extending vertically from the bundle sheath of minor veins to the upper and lower epidermal layers or to morphologically modified cells in the palisade.

The study of the vascularization patterns was confined primarily to the cleared leaf samples. Initial intervascular interval measurements were made from the paradermal sections, but the cleared tissue samples were used to corroborate these data. For the purpose of this study, unless otherwise noted, the intervascular interval is considered as the distance from a vein ending to the nearest minor vein perpendicular to the vein ending. For each species, a minimum of 30 intervascular interval measurements were taken from the cleared tissue samples using an ocular micrometer at 80X. After converting the measurements to micron values, the mean intervascular

interval value for each species was calculated. These mean values were compared with similar data from the paradermal sections which represented mean intervascular interval values from a minimum of 80 measurements made at 100X.

While the preceding aspects of foliar organization were being observed, measured, and recorded, notes were made on qualitative cytological and anatomical features as they were observed. Photomicrographs were later taken of some of these features.

Plate 1

Figure 1: Ranunculus acris, x.s. leaf (X 2000).

Figure 2: R. acris, paradermal section through mesophyll (X 600).

Figure 3: R. acris v. latisectus, x.s. leaf (X 2000).

Figure 4: R. acris v. latisectus, paradermal section through palisade (X 600).

Figure 5: R. repens, x.s. leaf (X 2000).

Figure 6: R. septentrionalis, x.s. leaf (X 1700).

Figure 7: R. pensylvanicus, x.s. leaf (X 1600).

Figure 8: R. hispidus, x.s. leaf (X 1500).

Figure 9: R. ficaria, x.s. leaf (X 2000).

Explanation of Symbols

P = palisade mesophyll cell; I = intercellular space.

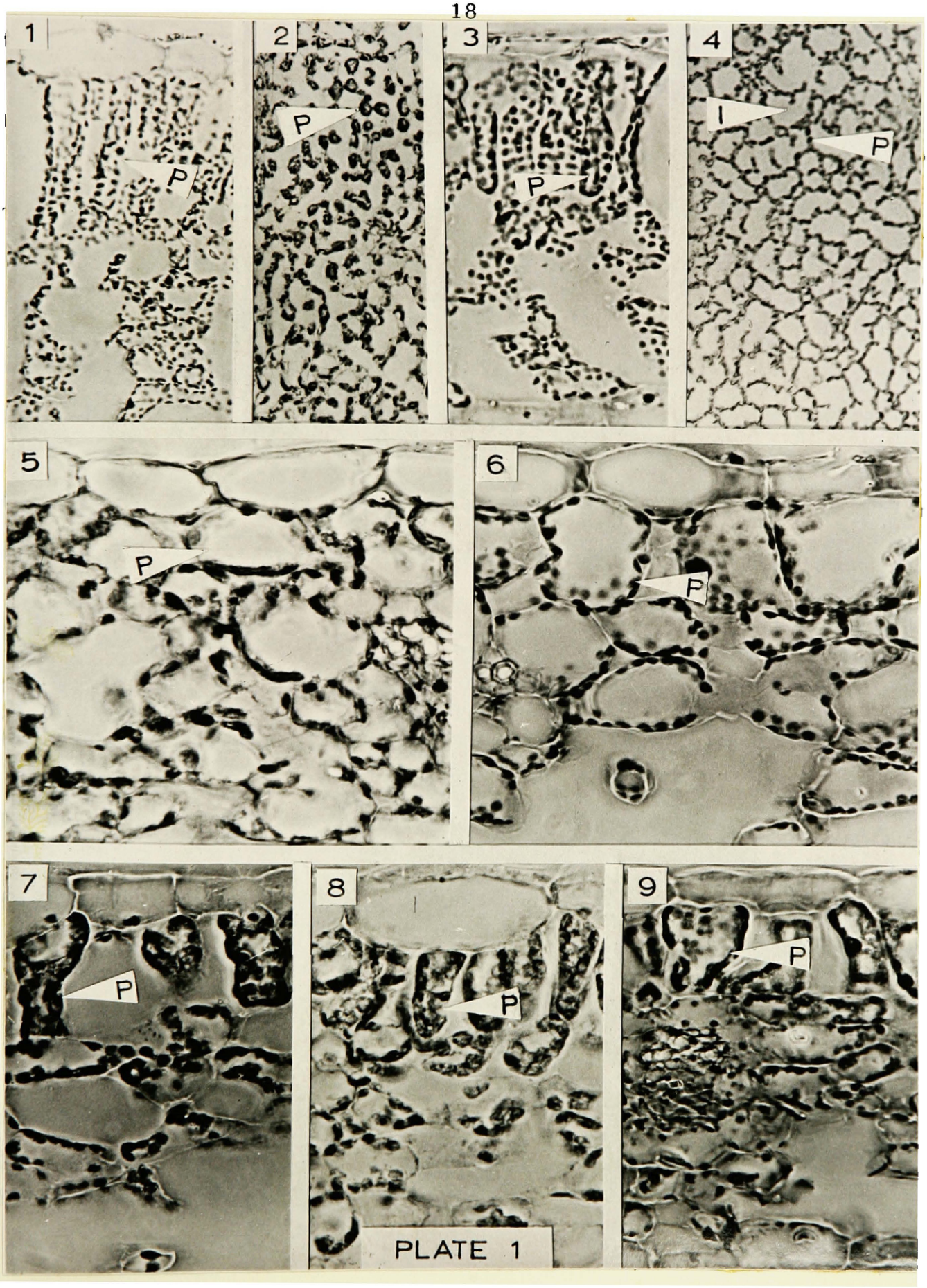

PLATE 1

Plate 2

Figure 1: Hepatica americana, x.s. leaf (X 2000).

Figure 2: Clematis verticillaris, x.s. leaf (X 1900).

Figure 3: Paeonia albiflora, x.s. leaf (X 2000).

Figure 4: P. albiflora, paradermal section through palisade (X 700).

Figure 5: Thalictrum polygamum, x.s. leaf (X 2100).

Figure 6: T. polygamum, paradermal section through mesophyll (X 600).

Figure 7: Caltha palustris, x.s. leaf (X 1900).

Figure 8: C. palustris, paradermal section through mesophyll (X 600).

Figure 9: Coptis groenlandica, x.s. leaf (X 2400).

Figure 10: Aquilegia canadensis, x.s. leaf (X 1900).

Figure 11: Coptis groenlandica, paradermal section through palisade (X 600).

Figure 12: Aquilegia canadensis, paradermal section through palisade (X 600).

Figure 13: Delphinium tricorne, x.s. leaf (X 1500).

Figure 14: D. tricorne, paradermal section through palisade (X 600).

Explanation of Symbols

F = flanged palisade cell; Fu = funnel palisade; C = compact palisade contiguous with the upper epidermis in Thalictrum polygamum; As = air space in the palisade mesophyll of Caltha palustris; Mp = multiple palisade; Sp = spongy mesophyll; B = bundle sheath; Be = bundle sheath extension; P = palisade; I = intercellular space.

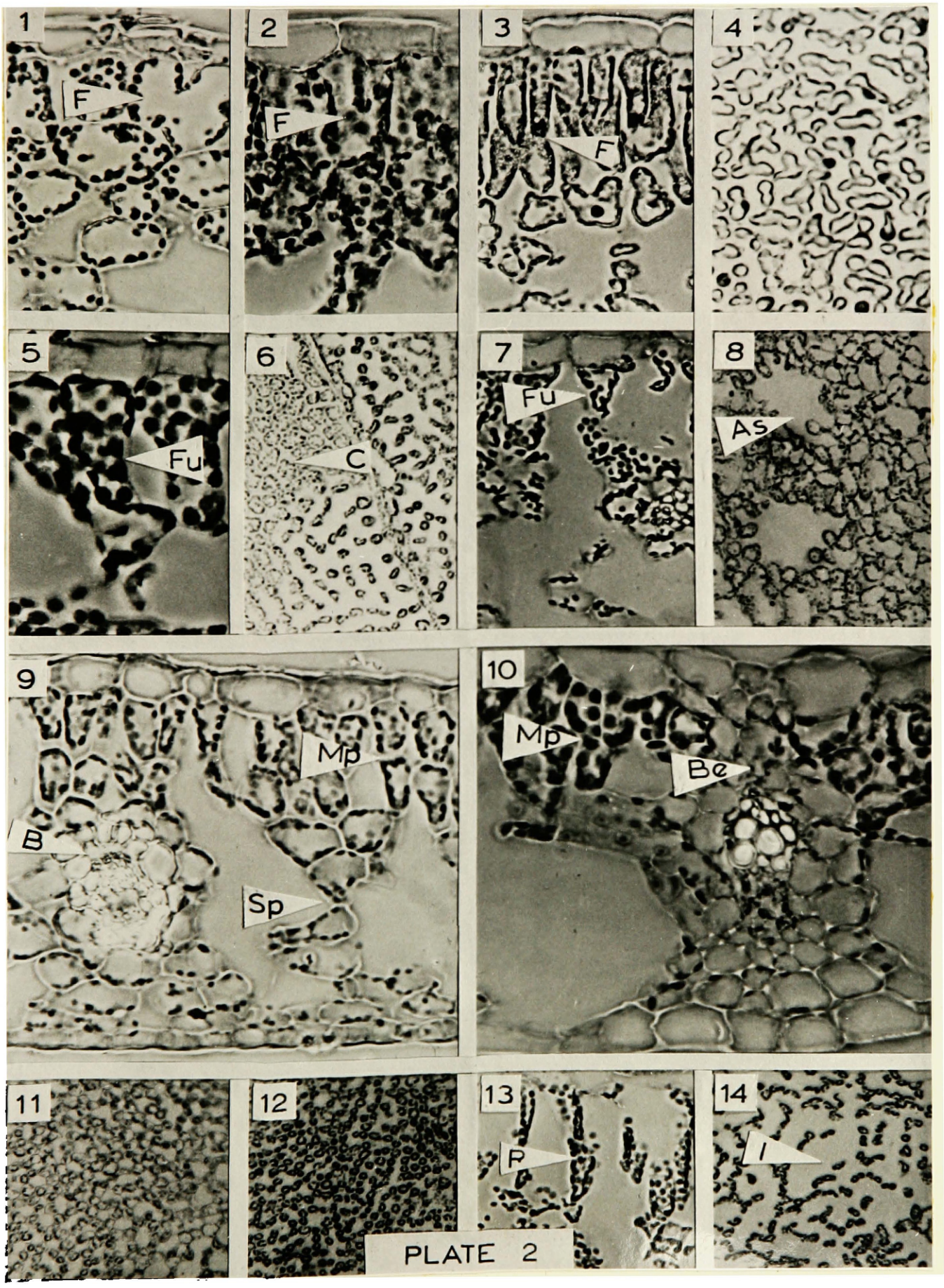

PLATE 2

Plate 3

Figure 1: Hepatica americana, x. s. leaf (X 2000).

Figure 2: H. americana, paradermal section through compact spongy mesophyll (X 600).

Figure 3: Ranunculus hispidus, paradermal section through palisade (top) and spongy (bottom) mesophyll (X 600).

Figure 4: R. repens, paradermal section through spongy mesophyll (X 600).

Figure 5: Cimicifuga racemosa, paradermal section through spongy mesophyll (X 600).

Figure 6: Anemone cylindrica, paradermal section through spongy mesophyll (X 600).

Explanation of Symbols

I = intercellular space; Sp = spongy mesophyll cell.

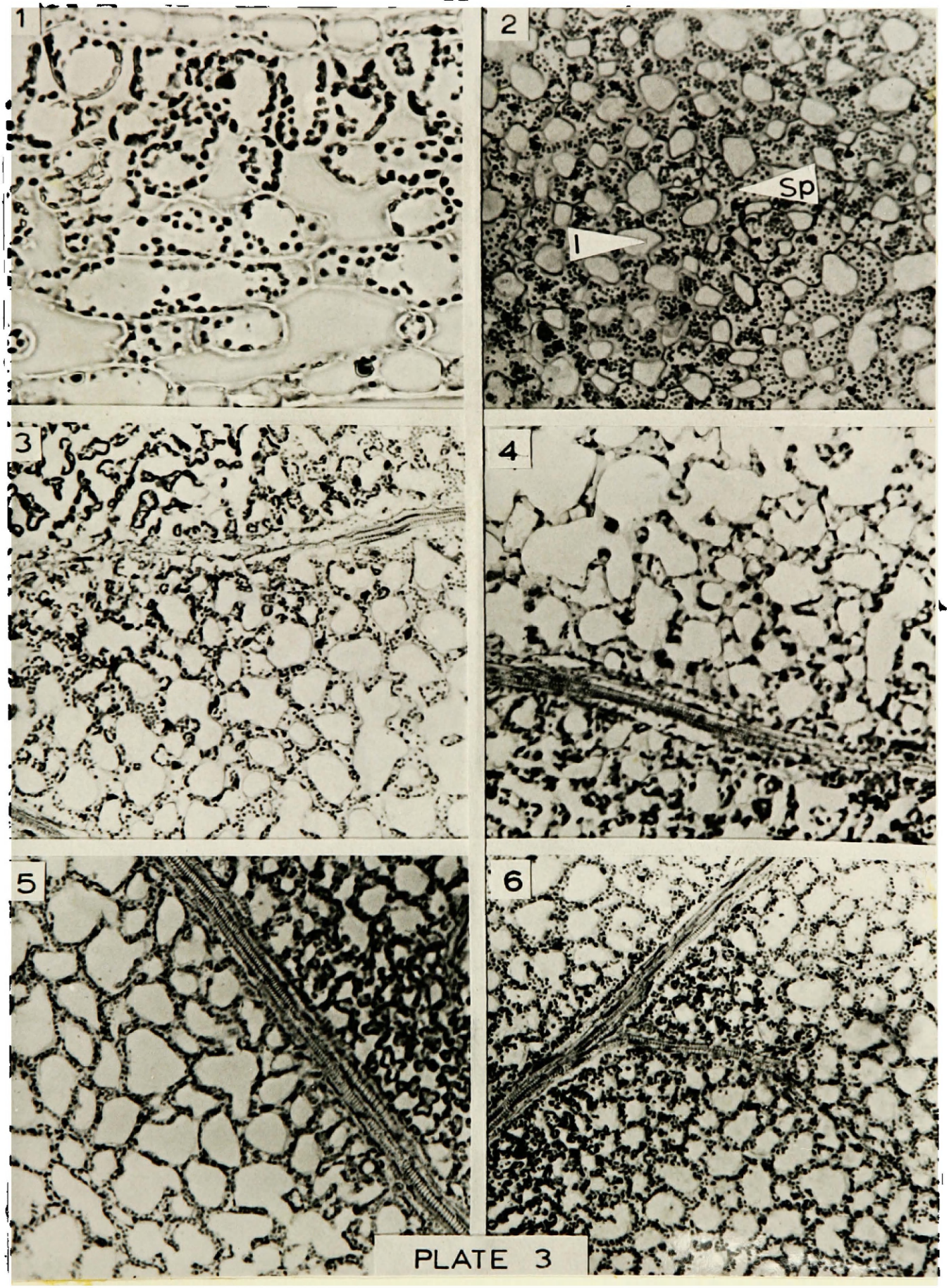

PLATE 3

RESULTS

Qualitative Observations on Internal Structure

In the genus Ranunculus (Plate 1), elongated, tubular palisade cells occurred in R. acris (Fig. 1) and R. acris v. latisectus (Fig. 3). Although elongated, the palisade tissue in these species is loosely packed, evident by its appearance in paradermal section (Figs. 2 and 4). The palisade in R. repens (Fig. 5) was poorly developed. A palisade region is distinguishable in R. septentrionalis (Fig. 6) but there is little elongation, the cells appearing to be isometric in shape. In R. pensylvanicus (Fig. 7), R. hispidus (Fig. 8), and R. ficaria (Fig. 9) there is some elongation of palisade cells and a palisade region is evident. The tissue is least compact in R. pensylvanicus, and of the three species, appears to be best developed in R. ficaria.

Among the other genera (Plate 2), flanged palisade was observed in Hepatica, Clematis, and was especially evident in Paeonia (Figs. 1, 2, and 3 respectively). The paradermal appearance of flanged palisade in Paeonia can be seen in Figure 4. The extensive development of palisade in Thalictrum polygamum can be noted from Figure 5. This species and Caltha palustris (Fig. 7) had funnel-shaped palisade. In Figure 6, which shows the paradermal appearance of the palisade region in Thalictrum polygamum, the compactness of this

tissue where it is contiguous with the upper epidermis can be noted. Further inward from the epidermis the amount of intercellular space increases as the diameter of the palisade cells decreases. The large air spaces in the palisade region of Caltha palustris (Fig. 8) were a unique feature of palisade organization in this study. A multiple palisade developed in Coptis groenlandica (Fig. 9) and Aquilegia canadensis (Fig. 10). Lateral contiguity of palisade cells was observed in Coptis from the paradermal sections (Fig. 11). In Aquilegia (Fig. 12), the palisade cells were observed to be close together but with few lateral contacts. Tubular palisade cells, comparable to those of Ranunculus acris and R. acris v. latisectus (Plate 1; Figs. 1 and 3) were observed in Delphinium tricorne (Fig. 13). Paradermal sections (Fig. 14) showed large intercellular spaces and very little contiguity between cells of the palisade region in this species.

The compactness of the spongy mesophyll in Hepatica was evident in the transverse sections (Plate 3; Fig. 1) but especially apparent in the paradermal sections (Fig. 2) where it was observed to form a continuous cellular net in which the intercellular spaces are relatively small. This condition in Hepatica can be contrasted with paradermal configurations of spongy mesophyll observed in Ranunculus hispidus (Fig. 3), R. repens (Fig. 4), Cimicifuga racemosa (Fig. 5), and Anemone cylindrica (Fig. 6), representative of the varying degrees

of compactness of this tissue observed in the study. The vertical alignment of spongy mesophyll cells in Coptis groenlandica (Plate 2; Fig. 9) was unique.

Quantitative Analyses of Internal Structure

From a preliminary investigation of the transverse sections it was noted that the arrangement of tissues in the leaf was atypically distorted in the regions where vascular bundles were present. In view of this, measurements of tissue components were made at intervascular areas.

The measurement data for total blade thickness and thickness of component blade tissues appear on Table I. For each species, the measurements on the top line represent species mean values. The measurements on the second and third lines, respectively, represent mean values for the thinnest and thickest leaf samples.

TABLE I. --Total blade thickness, thicknesses of the upper epidermis (UE), palisade (Pal), spongy mesophyll (Sp. M), and lower epidermis (LE) of the species mean, thinnest, and thickest samples in descending order. Measurement data in microns.

Species	Total	UE	Pal	Sp. M	LE
Delphinium tricorne	256.0	24.0	94.4	116.8	20.8
	203	19	80	86	18
	326	27	98	179	22
Ranunculus acris	251.2	28.8	86.4	118.4	17.6
	238	29	83	109	18
	269	30	86	133	18

TABLE I--Continued.

Species	Total	UE	Pal	Sp. M	LE
Caltha palustris	233.6	22.4	49.6	145.6	16.0
	187	19	43	110	14
	266	29	50	168	19
Ranunculus pensylvanicus	203.2	24.0	36.8	124.8	19.2
	162	21	37	87	16
	230	29	33	149	18
Hepatica americana	198.4	27.2	41.6	105.6	24.0
	165	24	34	85	22
	238	29	43	141	26
Paeonia albiflora	195.2	17.6	76.8	86.4	14.4
	157	19	58	64	16
	246	18	109	106	14
Ranunculus septentrionalis	193.6	25.6	38.4	112.0	17.6
	168	21	35	96	16
	243	27	48	149	19
Ranunculus acris v. latisectus	174.4	22.4	51.2	83.2	17.6
	150	19	46	69	16
	222	19	85	99	19
Ranunculus hispidus	163.2	22.4	28.8	96.0	16.0
	140	19	30	76	15
	181	25	29	110	16
Hepatica acutiloba	163.2	22.4	33.6	86.4	20.8
	128	18	27	66	18
	197	26	40	107	24
Coptis groenlandica	161.6	17.6	32.0	97.6	14.4
	133	16	29	75	13
	182	19	32	115	16
Ranunculus repens	150.4	19.2	28.8	88.0	14.4
	126	19	18	74	16
	220	22	37	146	15

TABLE I--Continued.

Species	Total	UE	Pal	Sp. M	LE
Ranunculus ficaria	144.0	23.0	40.8	64.2	16.0
	125	20	36	51	17
	164	23	35	90	16
Clematis verticillaris	137.6	20.8	41.6	55.6	17.6
	109	18	29	46	16
	203	27	74	82	21
Cimicifuga racemosa	116.8	14.4	28.8	59.2	14.4
	104	13	26	53	13
	126	14	38	61	13
Anemone cylindrica	112.0	24.0	25.6	43.2	19.2
	91	19	22	34	16
	154	34	26	64	30
Aquilegia canadensis	110.4	16.0	24.0	56.0	14.4
	101	18	22	48	13
	128	16	26	72	14
Actaea pachypoda	107.2	19.2	25.6	46.4	16.0
	96	19	27	35	14
	117	21	26	54	16
Thalictrum polygamum	107.2	14.4	43.2	36.8	12.8
	96	14	37	34	13
	117	16	50	38	13
Anemone virginiana	100.8	19.2	27.2	36.8	17.6
	85	19	22	27	16
	115	19	30	48	18

The largest sample variability occurred in Delphinium tricorne where the difference between the thinnest and thickest leaves was 123 microns. The smallest sample variability was found in the species

Actaea pachypoda and Thalictrum polygamum. Thinnest and thickest leaf samples in these species differed by 21 microns. The average difference in thickness between thin and thick leaf samples in the study was 59 microns.

Regarding component blade tissues, on the basis of the mean measurements for the species, the lower epidermis showed the least amount of variation, ranging in thickness from 24 microns in Hepatica americana to 12.8 microns in Thalictrum polygamum. Upper epidermal mean values ranged from 28.8 microns in Ranunculus acris to 14.4 microns in Thalictrum polygamum and Cimicifuga racemosa. Greatest variation was found in the tissues of the mesophyll. The palisade ranged from 94.4 microns in Delphinium tricorne to 24.4 microns in Aquilegia canadensis. The spongy mesophyll, the most variable blade tissue component, showed a range from 145.6 microns in Caltha palustris to 36.8 microns in Thalictrum polygamum and Anemone virginiana.

In order to better evaluate the actual measurement data on Table I, the relative percentages of the blade tissue components were calculated. This facilitated comparing the blade composition between species. The calculated relative percentages of tissues appear on Table II. The rank is in descending order of the total percentage of mesophyll tissue; i.e., a summation of the percentages of palisade and spongy mesophyll. The top line represents relative percentages

derived from the measurement data for the sample mean. The relative percentages of palisade and spongy mesophyll only are presented for the thinnest and thickest leaf samples in the second and third lines, respectively.

TABLE II.--Relative percentages of blade tissue components presented for the species mean; relative percentages of mesophyll tissues presented for thinnest and thickest samples. Rank is in descending order according to the total percentage of mesophyll tissues. Abbreviations as in Table I.

Species	UE	Pal	Sp. M	LE
Caltha palustris	9.5%	21.2% 23 19	62.3% 60 64	6.8%
Paeonia albiflora	9.0	39.3 38 44	43.4 42 43	7.3
Delphinium tricorne	9.3	36.8 39 30	45.6 43 55	8.1
Ranunculus acris	11.4	34.3 35 32	47.1 46 49	7.0
Coptis groenlandica	10.8	19.8 21 18	60.3 56 64	8.9
Ranunculus pensylvanicus	11.8	18.1 23 15	61.4 54 65	9.4
Ranunculus septentrionalis	13.3	20.0 21 20	58.3 57 61	9.1

TABLE II--Continued.

Species	UE	Pal	Sp. M	LE
Ranunculus repens	12.7%	19.1% 14 17	58.5% 58 66	9.5%
Ranunculus hispidus	13.7	18.6 22 16	58.8 54 61	9.8
Ranunculus acris v. latisectus	12.8	29.3 31 38	47.7 46 45	10.0
Cimicifuga racemosa	12.3	24.6 25 30	50.6 51 48	12.3
Hepatica americana	13.0	21.1 21 18	53.6 52 59	12.1
Thalictrum polygamum	13.4	40.2 38 43	34.3 35 33	11.9
Ranunculus ficaria	15.5	28.8 30 22	44.4 41 55	11.1
Hepatica acutiloba	13.5	20.3 21 20	52.4 51 54	12.6
Aquilegia canadensis	14.4	21.7 22 20	50.7 48 56	13.0
Clematis verticillaris	14.9	29.8 26 36	41.3 43 40	12.6

TABLE II--Continued.

Species	UE	Pal	Sp. M	LE
Actaea pachypoda	17.9%	23.8% 28 22	43.2% 37 47	14.9%
Anemone virginiana	19.0	26.9 26 26	36.5 31 42	17.4
Anemone cylindrica	21.4	22.8 25 17	38.5 37 42	17.1

From an analysis of the data on Table II, it was noted that the species under study showed definite patterns of mesophyll organization which were especially apparent between thin and thick leaf samples of each species. There was a difference in the magnitude of mesophyll development and also in the proportional development of palisade and spongy mesophyll components. The species were grouped according to how much variation was observed in the relative percent of mesophyll tissues in the thinnest and thickest samples when compared to the species mean values. On this basis, four groups were set up. In Group 1, the relative percent of the mesophyll tissues was identical for the thick and thin samples and the species mean. In Group 2, the relative percent of mesophyll in the thick and thin samples varied from the species mean by $\pm$ 2%. In Group 3, this variation from the

mean values was ± 3-4%, and in Group 4, the relative percent of mesophyll tissues varied from the mean values by more than ± 4%. Within each group the mesophyll components of the thick and thin leaf samples were analyzed. Their proportional development was expressed in percentage deviation from the sample mean values. The results of this analysis follow:

Group 1: No variation from the sample mean in relative percentage of mesophyll for thin and thick samples.

Caltha palustris
thin leaf: relative % palisade +2%; relative % spongy mesophyll -2%.
thick leaf: rel. % pal. -2%; rel. % sp. m. +2%
Ranunculus acris
thin leaf: rel. % pal. +1%; rel. % sp. m. -1%
thick leaf: rel. % pal. -2%; rel. % sp. m. +2%

Group 2: Relative % mesophyll = mean value ± 2%

Delphinium tricorne
thin leaf: rel. % pal. +2%; rel. % sp. m. -3%
thick leaf: rel. % pal. -7%; rel. % sp. m. +9%
Ranunculus pensylvanicus
thin leaf: rel. % pal. +5%; rel. % sp. m. -7%
thick leaf: rel. % pal. -3%; rel. % sp. m. +4%
Actaea pachypoda
thin leaf: rel. % pal. +4%; rel. % sp. m. -6%
thick leaf: rel. % pal. -2%; rel. % sp. m. +4%
Hepatica americana
thin leaf: rel. % pal. =$\bar{m}$%; rel. % sp. m. -2%
thick leaf: rel. % pal. - 3%; rel. % sp. m. +5%
Hepatica acutiloba
thin leaf: rel. % pal. +1%; rel. % sp. m. -1%
thick leaf: rel. % pal. =$\bar{m}$%; rel. % sp. m. +2%
Ranunculus hispidus
thin leaf: rel. % pal. +3%; rel. % sp. m. -5%
thick leaf: rel. % pal. -3%; rel. % sp. m. +2%

Cimicifuga racemosa
thin leaf: rel. % pal. =$\bar{m}$%; rel. % sp. m. = $\bar{m}$%
thick leaf: rel. % pal. +5%; rel. % sp. m. -3%
Thalictrum polygamum
thin leaf: rel. % pal. -2%; rel. % sp. m. +1%
thick leaf: rel. % pal. +3%; rel. % sp. m. -1%

Group 3: Relative % mesophyll = mean value ± 3-4%

Anemone cylindrica
thin leaf: rel. % pal. +2%; rel. % sp. m. -2%
thick leaf: rel. % pal. -6%; rel. % sp. m. +3%
Ranunculus septentrionalis
thin leaf: rel. % pal. +1%; rel. % sp. m. -1%
thick leaf: rel. % pal. =$\bar{m}$%; rel. % sp. m. +3%
Ranunculus ficaria
thin leaf: rel. % pal. +1%; rel. % sp. m. -3%
thick leaf: rel. % pal. -7%; rel. % sp. m. +11%
Aquilegia canadensis
thin leaf: rel. % pal. =$\bar{m}$%; rel. % sp. m. -3%
thick leaf: rel. % pal. -2%; rel. % sp. m. +5%
Coptis groenlandica
thin leaf: rel. % pal. +1%; rel. % sp. m. -4%
thick leaf: rel. % pal. -2%; rel. % sp. m. +4%
Paeonia albiflora
thin leaf: rel. % pal. -2%; rel. % sp. m. -1%
thick leaf: rel. % pal. +4%; rel. % sp. m. =$\bar{m}$%

Group 4: Relative % mesophyll = mean value ± more than 4%

Anemone virginiana
thin leaf: rel. % pal. -1%; rel. % sp. m. -6%
thick leaf: rel. % pal. -1%; rel. % sp. m. +5%
Ranunculus repens
thin leaf: rel. % pal. -5%; rel. % sp. m. -1%
thick leaf: rel. % pal. -2%; rel. % sp. m. +7%
Clematis verticillaris
thin leaf: rel. % pal. -4%; rel. % sp. m. +2%
thick leaf: rel. % pal. +6%; rel. % sp. m. -1%
Ranunculus acris v. latisectus
thin leaf: rel. % pal. +2%; rel. % sp. m. -2%
thick leaf: rel. % pal. +9%; rel. % sp. m. -3%

The patterns of mesophyll organization apparent from this analysis are:

1) The relative percentage of mesophyll in the thin leaf is the same as or lower than the mean, the same as or higher than the mean in the thick leaf with thickness the result of greater spongy mesophyll development.

2) The relative percentage of mesophyll in the thin leaf is the same as or lower than the mean, higher than the mean in the thick leaf with thickness the result of greater palisade development.

3) The relative percentage of mesophyll in the thin leaf is the same as or lower than the mean, lower than the mean in the thick leaf with thickness the result of greater epidermal development.

The results of the computation of the tissue ratio for each species appear on Table III. It was noted that the tissue ratio is a means of expressing the relative proportions of component blade tissues which emphasizes the proportion of palisade tissue. In the computation of the tissue ratio it should be recalled that the volume of palisade tissue is the divisor. There is, therefore, a reciprocal relationship between the tissue ratio and the proportion of palisade. To show this, the species were arranged in ascending order according to the percentage of palisade tissue. This listing is on Table IV. With the exception of the positions of Coptis groenlandica and Ranunculus septentrionalis, the order is identical to that in Table III.

TABLE III.--Tissue ratios. The first value is the mean value for the species; second and third values represent the lowest and highest tissue ratios obtained from the ten leaf samples of each species studied. The rank is in descending order of the mean value.

Species	Mean	Low	High
Ranunculus pensylvanicus	4.8	3.1	6.9
Ranunculus hispidus	4.6	3.6	5.3
Ranunculus repens	4.4	3.1	6.1
Ranunculus septentrionalis	4.2	3.8	5.4
Coptis groenlandica	4.0	3.6	4.7
Hepatica acutiloba	3.8	3.5	4.6
Hepatica americana	3.8	2.3	4.8
Caltha palustris	3.8	3.3	4.3
Aquilegia canadensis	3.6	2.8	4.4
Anemone cylindrica	3.4	2.8	4.8
Actaea pachypoda	3.2	2.5	3.9
Cimicifuga racemosa	3.1	2.1	3.8
Anemone virginiana	2.8	2.5	3.3
Ranunculus ficaria	2.6	2.1	3.7
Ranunculus acris v. latisectus	2.4	1.6	3.0
Clematis verticillaris	2.3	1.7	2.8
Ranunculus acris	1.9	1.6	2.1
Delphinium tricorne	1.7	1.5	2.3
Paeonia albiflora	1.6	1.3	1.8
Thalictrum polygamum	1.5	1.3	1.7

TABLE IV. -- Rank of species according to relative percentage palisade tissue, in ascending order.

Species	Percentage Palisade
Ranunculus pensylvanicus	18.1
Ranunculus hispidus	18.6
Ranunculus repens	19.1
Coptis groenlandica	19.8
Ranunculus septentrionalis	20.0
Hepatica acutiloba	20.3
Hepatica americana	21.1
Caltha palustris	21.2
Aquilegia canadensis	21.7
Anemone cylindrica	22.8
Actaea pachypoda	23.8
Cimicifuga racemosa	24.6
Anemone virginiana	26.9
Ranunculus ficaria	28.8
Ranunculus acris v. latisectus	29.3
Clematis verticillaris	29.8
Ranunculus acris	34.3
Delphinium tricorne	36.8
Paeonia albiflora	39.3
Thalictrum polygamum	40.2

Plate 4

Figure 1: Caltha palustris, cleared leaf sample (X 160).

Figure 2: Delphinium tricorne, cleared leaf sample (X 160).

Figure 3: Ranunculus pensylvanicus, cleared leaf sample (X 130).

Figure 4: Thalictrum polygamum, cleared leaf sample (X 130).

Figure 5: Clematis verticillaris, cleared leaf sample (X 130).

Figure 6: Ranunculus ficaria, cleared leaf sample (X 130).

Figure 7: Aquilegia canadensis, cleared leaf sample (X 160).

Figure 8: Hepatica acutiloba, cleared leaf sample (X 160).

Figure 9: H. americana, cleared leaf sample (X 150).

Figure 10: Paeonia albiflora, cleared leaf sample (X 150).

Figure 11: Coptis groenlandica, cleared leaf sample (X 150).

Explanation of Symbols

A = vein anastomosis; V = vein ending; B = bundle sheath.

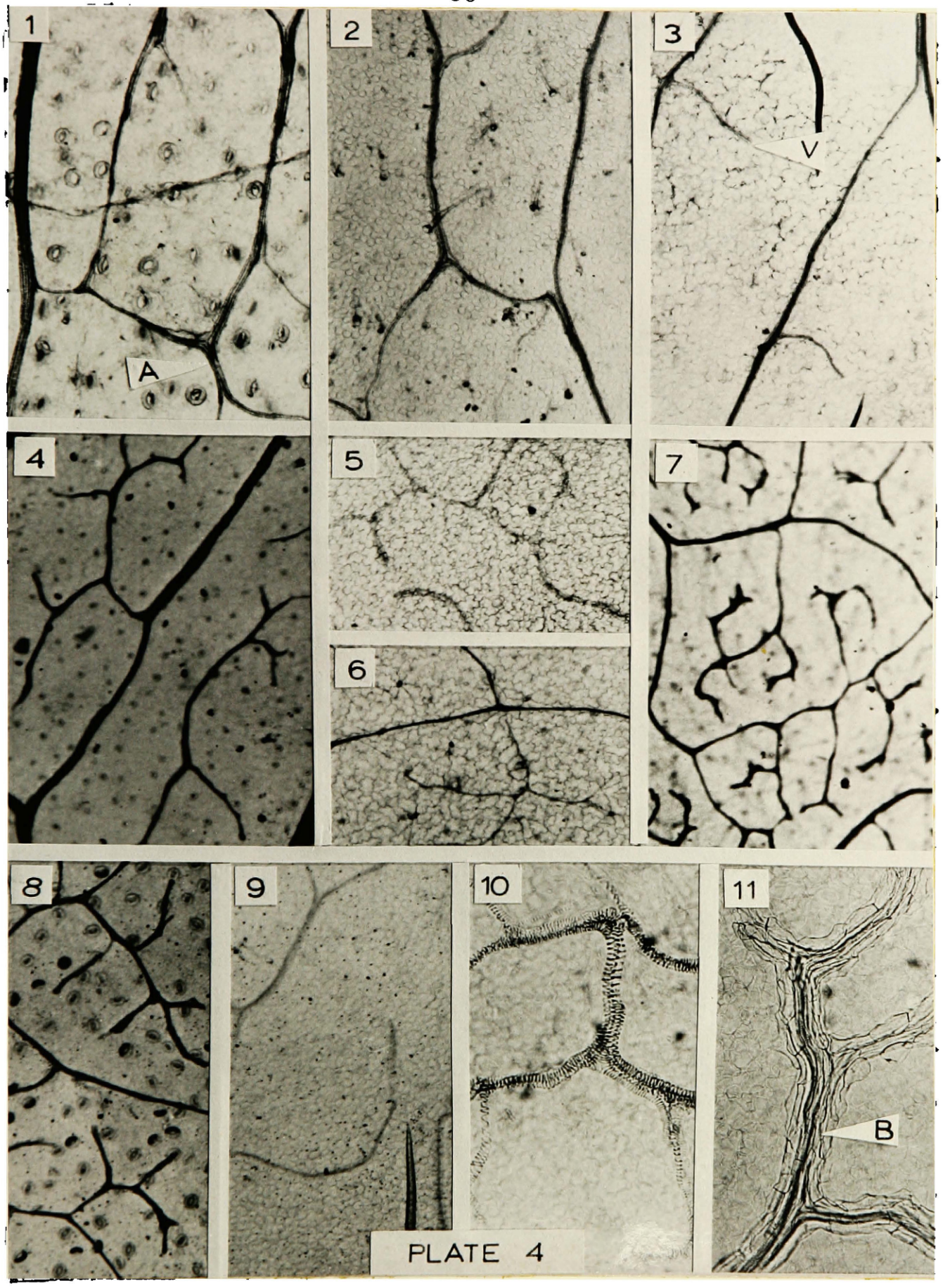

PLATE 4

Qualitative Observations on Venation

The venation patterns that were observed in this study are represented in the figures on Plate 4. Figures 1, 2, and 3 show the rectangular minor vein patterns of Caltha palustris, Delphinium tricorne, and Ranunculus pensylvanicus, respectively. It can be noted that there are few minor vein endings within each rectangular area. Vein anastomoses apparent in Caltha palustris (Fig. 1) were also observed in Cimicifuga racemosa.

Thalictrum polygamum (Fig. 4) and Clematis verticillaris (Fig. 5) had a scorpioid minor vein pattern. In the former species the mean distance between parallel minor veins was similar to the mean distance from minor vein ending to the nearest vein perpendicular to the vein ending. This can be seen from the representative venation area in Figure 4.

Figure 6 shows the extent of some minor veins composed of a single tracheid observed in Ranunculus ficaria.

The dendroid venation pattern of Aquilegia canadensis is represented in Figure 7. The close network of minor veins is evident.

Figures 8 and 9 show the extremes in venation observed in the two species of Hepatica. In Hepatica acutiloba (Fig. 8) some leaf samples had venation patterns like that pictured for H. americana (Fig. 9) and likewise, some samples of H. americana showed the type pictured for H. acutiloba.

Paeonia albiflora (Fig. 10) and Coptis groenlandica (Fig. 11) lacked a definite pattern of venation. A bundle sheath of fibers and parenchyma cells can be seen associated with the minor veins of Coptis.

Quantitative Analyses of Venation

The criteria followed by other investigators for obtaining intervascular interval measurements were not altogether apparent in the literature. After two sets of data were compiled from the paradermal sections, it was noted that the distance from a vein ending to the nearest vein perpendicular to the vein ending was more consistent for a given species than the distance between any two parallel minor veins. In Paeonia, Ranunculus acris v. latisectus, Aquilegia, and Thalictrum, the venation pattern was such that both means of measuring resulted in similar intervascular interval values with the standard deviation the micron equivalent of approximately 42, 49, 35, and 42 respectively. For Ranunculus hispidus, R. ficaria, R. septentrionalis, R. repens, and Actaea, intervascular interval measurements from parallel minor veins resulted in standard deviations (in microns) of approximately 77, 63, 91, 84, and 77 respectively. When the distance from vein ending to the nearest vein was measured, the standard deviations were reduced to approximately 35, 35, 56, 36, and 35 (micron equivalents) respectively. Hepatica americana and H. acutiloba had vascular

patterns such that both means of measuring resulted in a wide range of intervascular interval values for the species with standard deviations of 84 microns and 77 microns respectively (Plate 4, Figs. 8 and 9). Within any one leaf, however, the distance from vein ending to the nearest vein was more consistent.

For nine of the species no intervascular interval data were obtained from the paradermal sections since they contained only fragmentary portions of mesophyll showing vascular tissue. It would have been necessary to construct and interpret a composite of serial sections which could not have been done completely objectively. Two complete sets of intervascular interval measurements were therefore compiled from the cleared leaf portions. These data corroborated those from the paradermal sections. The only species for which the distance between parallel minor veins was measured for the determination of the intervascular interval was Delphinium tricorne. Data on the intervascular interval appear on Table V.

TABLE V.--Intervascular interval measurements presented in microns. The rank is in descending order.

Species	Mean intervascular interval value
Hepatica americana	356.9
Ranunculus pensylvanicus	323.7
Actaea pachypoda	302.0
Delphinium tricorne	293.0
Ranunculus hispidus	287.6
Ranunculus septentrionalis	278.0
Hepatica acutiloba	237.0
Ranunculus repens	221.0
Anemone virginiana	205.7
Caltha palustris	202.0
Anemone cylindrica	186.0
Cimicifuga racemosa	177.0
Ranunculus ficaria	165.2
Coptis groenlandica	159.3
Ranunculus acris	157.8
Ranunculus acris v. latisectus	155.7
Thalictrum polygamum	151.0
Clematis verticillaris	147.5
Paeonia albiflora	134.0
Aquilegia canadensis	81.0

Plate 5

Figure 1: Cimicifuga racemosa, x.s. leaf (X 3100).

Figure 2: Clematis verticillaris, x.s. leaf (X 1900).

Figure 3: Thalictrum polygamum, x.s. leaf (X 2100).

Figure 4: Actaea pachypoda, x.s. leaf (X 2200).

Figure 5: Ranunculus ficaria, x.s. leaf (X 2000).

Figure 6: Anemone cylindrica, x.s. leaf (X 2300).

Figure 7: Caltha palustris, x.s. leaf (X 1900).

Figure 8: Delphinium tricorne, x.s. leaf (X 1500).

Explanation of Symbols

Be = bundle sheath extension; B = bundle sheath.

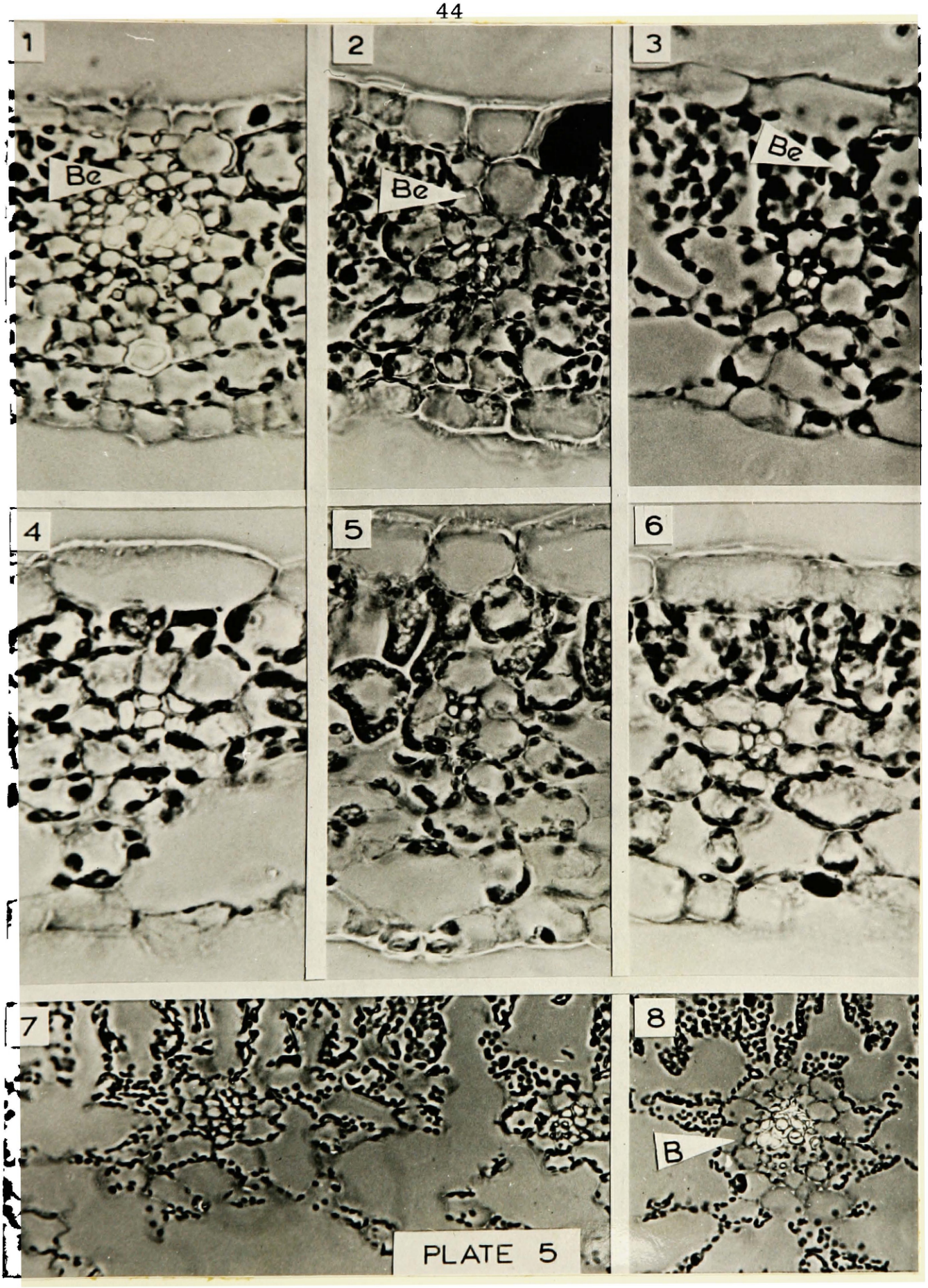

PLATE 5

Qualitative Observations on the Bundle Sheath Extension

On Plate 5, the appearance of the bundle sheath extension associated with the minor veins of Cimicifuga racemosa (Fig. 1), Clematis verticillaris (Fig. 2), and Thalictrum polygamum (Fig. 3) can be seen. (Plate 2, Fig. 10 shows a well-developed bundle sheath extension in Aquilegia canadensis.)

Figures 4, 5, and 6 show minor veins of Actaea pachypoda, Ranunculus ficaria, and Anemone cylindrica, respectively, without sheath extensions. The fibrous and parenchyma bundle sheath of Coptis groenlandica which lacks extensions can be observed on Plate 2, Figure 9.

Figure 7 on Plate 5 shows two minor veins lacking extensions as they were observed in Caltha palustris. Note that the wide bundle sheath of Delphinium tricorne (Fig. 8) lacks the sheath extension.

Quantitative Analyses of the Bundle Sheath Extension

For seven of the species in this study there was no evidence of the occurrence of the bundle sheath extension with minor veins. These species were: Delphinium tricorne, Caltha palustris, Ranunculus pensylvanicus, Hepatica americana, H. acutiloba, Paeonia albiflora, and Coptis groenlandica. In ten species, less than 50% of the minor veins observed had associated bundle sheath extensions. When more than one minor vein with a sheath extension was observed in a

transverse section, the distance between the veins was measured and the mean distance between minor veins with sheath extensions was calculated for the species. A summary of the results appears on Table VI.

TABLE VI.--Frequency and spacing of minor veins with bundle sheath extensions. Distance between minor veins with bundle sheath extensions (bse) presented in microns.

Species	Mean distance	Remarks
Ranunculus hispidus	1858	bse observed in only 3 leaf samples
Actaea pachypoda	1534	measurable distances on 4 samples; 2 samples contained 1 vein with bse
Ranunculus ficaria	1430.7	bse present in 9 of 10 leaf samples
Ranunculus repens	1401	bse in 8 of 10 samples; 2 thickest samples lacked bse with minor veins
Cimicifuga racemosa	1333.4	bse present in 6 of 10 samples
Ranunculus septentrionalis	1091	bse observed in 2 thinnest samples
Ranunculus acris	1032	bse associated with minor veins in 4 of 10 samples
Ranunculus acris v. latisectus	850	bse associated with minor veins in 2 thinnest samples

TABLE VI--Continued.

Species	Mean distance	Remarks
Anemone virginiana	678	approximately 40% of minor veins with bse
Anemone cylindrica	634	approximately 40% of minor veins with bse
Aquilegia canadensis	1224.3	approximately 60% of minor veins with bse
Clematis verticillaris	998.6	frequency of bse with minor veins greater than 50%; on 2 thinnest samples mean distance between minor veins with bse = 708
Thalictrum polygamum	678.5	approximately 90% of minor veins with bse

It should be emphasized that the distances recorded in Table VI are distances between minor veins having associated sheath extensions and not the distances between minor veins in general.

Since the occurrence of bundle sheath extensions has been associated with spongy mesophyll development in other studies, the species under consideration were arranged in descending order according to the relative percentage of this tissue. This listing is in Table VII.

TABLE VII. --Rank of species in descending order according to the relative percentage of spongy mesophyll tissue.

Species	Percentage Spongy Mesophyll
Caltha palustris	62.3
Ranunculus pensylvanicus	61.4
Coptis groenlandica	60.3
Ranunculus hispidus	58.8
Ranunculus repens	58.5
Ranunculus septentrionalis	58.3
Hepatica americana	53.6
Hepatica acutiloba	52.4
Aquilegia canadensis	50.7
Cimicifuga racemosa	50.6
Ranunculus acris v. latisectus	47.7
Ranunculus acris	47.1
Delphinium tricorne	45.6
Ranunculus ficaria	44.4
Paeonia albiflora	43.4
Actaea pachypoda	43.2
Clematis verticillaris	41.3
Anemone cylindrica	38.5
Anemone virginiana	36.5
Thalictrum polygamum	34.3

In examining the slides, which were serial sections of each leaf sample, it was noted that the bundle sheath extension was not always a constant feature of a minor vein, being visible in some transverse sections but not in others. There was also evidence of incomplete bundle sheath extensions; i.e., extensions that were contiguous with either upper or lower epidermal layers but not both. In cases where the sheath extension was incomplete to the upper epidermis, no modified palisade cells were observed which could have functioned to complete the extension. Neither of these conditions has been reported in other studies on foliar organization.

In leaf samples which lacked bundle sheath extensions associated with minor veins, numerous minor veins were nevertheless present in the transverse sections (Plate 5, Fig. 7).

DISCUSSION

Within any of the twenty species under study, neither the habitat of the plants nor the amount of insolation received by the leaves was a variable. In this study there are sun and shade species, but no sun and shade leaves. The concept that sun leaves are thick and shade leaves are thin must be interpreted in reference to leaves on a given plant receiving different amounts of insolation. The quantity of experimental work done on sun and shade leaves which substantiates this (Anderson, 1955; Shields, 1949; Wylie, 1951) is not being questioned. The fact that a plant grows under conditions of high insolation does not imply, however, that its leaves will be thicker than those of another species which grows in partial or full shade. As it was shown in this study, of the five sun species, Delphinium tricorne and Ranunculus acris have the thickest leaves of the species studied. The other three sun species, however, Ranunculus acris v. latisectus, R. ficaria, and Clematis verticillaris, are ranked 8, 13, and 14 in total blade thickness (Table I). Sinnott (1960) emphasized that the genotype and phenotype are both manifested in ultimate plant structure. The phenotype may be modified by environmental conditions but the genotype determines the extent to which this modification can occur. Relative to actual blade thickness in this study, genetic variation between species is probably involved more than environmental conditions.

Within the genus Ranunculus alone, average blade thickness varied from 251.2 microns in R. acris to 144.0 microns in R. ficaria, and there was no correlation between blade thickness and habitat.

The method used to obtain leaf samples was devised in order to minimize the variability in leaf structure due to position on the shoot (Zalenski, 1904 in Shields, 1949; Clowes, 1956) and differential maturation rates of tissues in a given leaf (Maksymowych, 1959). The average difference in thickness between the thinnest and thickest leaf samples in the study was 59 microns. Four of the species had what were considered unusually large differences in thickness between thin and thick samples. These were Delphinium tricorne (123 microns), Clematis verticillaris and Ranunculus hispidus (94 microns), and Paeonia albiflora (89 microns). There is no apparent correlation between general habitat preference and the magnitude of difference between the thin and thick leaf samples. Of the sun species, three had a range in thickness greater than the average of 59 microns, two had thickness ranges below this figure. Fifteen species occur naturally in full or partial shade. Eight of these had above average variations in blade thickness, seven below. Of the seven species which have a preference for moist or wet habitats, four had above average thickness ranges, three had ranges below 59 microns.

By analyzing the relative percentage data on Table II, the magnitude of the expansion of the mesophyll was determined for the

species under study. There was no apparent correlation observed between blade thickness, the amount of difference between the thinnest and thickest leaf samples obtained for a species, and the amount of expansion of the mesophyll region.

The patterns of mesophyll organization observed when the palisade and spongy components were analyzed separately are most interesting. Thirteen of the twenty species have a mesophyll pattern that is considered typical of the dorsiventral mesophytic leaf: the relative percentage of mesophyll may remain constant as the leaf becomes thicker, but there is a higher relative percentage of palisade in the thin leaf, thickness resulting from greater development of the spongy mesophyll (Philpott, 1947; Wylie, 1951; Esau, 1953). Within the thirteen species exhibiting this trend, percentage increases in the spongy mesophyll over the mean values range from 2% in Caltha palustris, Ranunculus acris, and Hepatica acutiloba, to 11% in Ranunculus ficaria. Contrary to what is considered to be the typical mesophytic pattern of mesophyll development, five species in the current study showed a pattern of mesophyll organization in which expansion in the mesophyll region was due to an increase in the palisade component. This particular foliar pattern has been associated with sun leaves (Thompson, 1943; Isanogle, 1944; Wylie, 1951) and with xeric habitats (Shields, 1949; Daubenmire, 1947). Of the five species in which this

pattern was observed, Clematis verticillaris and Ranunculus acris v. latisectus are sun species. However, samples of Thalictrum polygamum and Cimicifuga racemosa were collected from plants growing in full shade, and the plants of Paeonia albiflora that were sampled were growing in partial shade. There are no xerophytes in this study; all twenty of the species are mesophytes. The habitats represented by the five species mentioned above vary from open meadow to wet, shaded woods. It is obvious from this study that there are factors other than high insolation and xeric growing conditions involved in palisade expansion. Cimicifuga racemosa in addition to being a shade species was sampled from plants growing on a creek bank in one of the wettest collection sites in the study. It is not being disputed that this foliar pattern is represented in sun leaves and leaves from xeric habitats. On the basis of this study it is being stated that not all sun leaves display this pattern, and that this pattern of mesophyll organization is not a peculiarity of sun and/or xeric species. Of the five species in which leaf thickness was attributed to palisade elongation, Thalictrum polygamum invites further study. In this species the percentage of palisade was greater than the percentage of spongy mesophyll in all of the leaves examined.

The third pattern of mesophyll organization in which the relative percentage of mesophyll was lower in the thick leaf than it was for

the species mean implies an expansion in the epidermal tissues. Ranunculus hispidus showed a decrease of 1% in the percentage of mesophyll; in Anemone cylindrica the decrease was 3%. In R. hispidus the epidermal tissues of the thick leaf account for 23% of the dorsiventral depth; they account for 41% of the depth in the thick leaf of Anemone cylindrica. In his work on the role of epidermis in foliar organization, Wylie (1943) found that the combined thickness of the epidermal layers averaged approximately 20% of the blade tissue between veins in forty-six species of herbaceous and woody dicotyledons. Wylie reported that since the epidermis is compact and the mesophyll has numerous intercellular spaces, the proportion of tissue in the epidermal layers is higher than its relative volume. In R. hispidus and Anemone cylindrica simple pits were observed in the radial walls of the cells of the upper epidermis which would facilitate lateral conduction through this tissue. The epidermis may function significantly in conduction in intervascular areas of the leaf.

Seven species of Ranunculus, two species of Hepatica, and two species of Anemone were among the twenty species in this study. Relative to their mesophyll organization, the two species of Hepatica were very similar; the two species of Anemone were dissimilar. Leaf morphology was not an apparent factor in this dissimilarity in that it is difficult to distinguish between Anemone virginiana and A. cylindrica

on the basis of leaf form. Taxonomically these two species are separated on flower and fruit characteristics. Both species of Anemone in this study were collected from similar habitats, being common understory species of white oak woods in northeastern Ohio. Hepatica americana and H. acutiloba have morphologically distinct leaves; the difference in the leaf apices is a minor difference, but it is sufficient to distinguish the species. The two species of Hepatica were collected from distinct sites. H. americana was sampled from a population on a dry slope in a white oak woods where its associates were Mitchella repens L. and Vaccinium vacillans Torr., two acid-soil indicator species in Ohio. H. acutiloba was collected from a population of plants in a beech-maple woods. Gray (1950) indicates that these two species of Hepatica are usually found in separate areas and lists sites such as those in which they were collected as typical. The species in the genus Ranunculus in this study vary in internal anatomy, habitat preference, and morphology.

The extent to which the relative percentage of spongy mesophyll varied in Coptis groenlandica (Table II) was not anticipated. This species is unique in the study in that it is an evergreen representative of the Ranunculaceae. Haberlandt (1914) indicated that evergreen leaves show a lack of "plasticity" in their development. Whether this statement was meant to apply to all evergreen leaves is not known; it

does not apply to Coptis in the current study. Coptis groenlandica is the one species in this study representative of a special habitat type. In northeastern Ohio it is confined in occurrence to bog environments and samples were collected from plants growing on a sphagnum shelf on the edge of a glacial bog. Philpott (1956) reported that an increase in leaf thickness of bog species was due to increased palisade development. However, palisade development in Coptis groenlandica was not extensive. The highest relative percent of palisade in any of the leaf samples was 21% in the thinnest leaf. Furthermore, it should be noted that Coptis groenlandica is among the thirteen species in which leaf thickness was due to spongy mesophyll expansion. Müller-Stoll (in Sinnott, 1960) associated xeromorphy with the nitrogen deficiency in bog soils. One aspect of xeromorphy directly associated with nitrogen deficiency according to Sinnott (1960) is the development of thick cell walls. Thickenings develop presumably because carbohydrate is deposited in cell walls which, when nitrogen is available, is used in protein synthesis. This is corroborated in the current study, Coptis groenlandica being the only species in which a fibrous bundle sheath was observed (Plate 4, Fig. 11).

The distribution of longitudinal and transverse growth in the leaf determines the course of the veins. The endless differences which appear in the venation of individual species of angiosperms

depend on: 1) the fact that the distribution of growth appears in its most varied expression in the leaf; 2) the different mass relationship between mesophyll and vein material in different plants (Goebel, 1922 in Foster, 1952). Venation patterns observed in this study can be seen on Plate 4.

Vein spacing has frequently been related to mesophyll organization. Mounts (1932) observed that the cells primarily concerned with the development of minor veins retain longest their capacity for normal cell division. This prolonged capacity aids the leaf in modifying vein patterns to the type of mesophyll developing. According to Armacost (1944), increased amounts of palisade mesophyll tend to force veins closer together while larger proportions of spongy mesophyll favor their wider separation. Recalling that the tissue ratio is a means of expressing mesophyll organization which emphasizes the role of the palisade, Philpott (1947) positively correlated tissue ratio and vein spacing (or intervascular interval) in forty-seven species of Ficus. According to Wylie (1946) the development of the palisade involves conduction problems proportional to its relative volume. Due to its arrangement at right angles to the plane of the leaf, palisade could only be retardive to lateral movement of materials in the leaf blade (Wylie, 1951).

Haberlandt (1914) reported a closer network of veins in leaves which transpire rapidly. Turrell (1936) concluded that extensive

palisade development increases the transpiration potential of leaves. Xerophytes characterized by extensive palisade development transpire more actively, when adequately supplied with water, than mesophytes (Kramer, 1949). Considering the effect of extensive palisade development on transpiration potential, then the correlation between tissue ratio and vein spacing seems logical. The correlations that have been reported have been based on studies of leaves with extensive palisade mesophyll: Ficus by Philpott (1947); leaves of bog species with low tissue ratios (Philpott, 1956); sun leaves of ten deciduous dicotyledon trees (Wylie, 1951). Wylie (1951) did find that in the shade leaves in his study in which the palisade development was retarded, the vascularization was not proportionately modified and could not be correlated with the tissue ratios of those leaves. A strict correlation between palisade development and the spacing of minor veins is not supported by the current study and is therefore not thought to be generally applicable as a principle of foliar organization. On the basis of the current investigation, the following remarks can be made relative to tissue ratio and the spacing of minor veins or intervascular interval:

1) Considering the study group as a whole, there is no apparent correlation between tissue ratio and intervascular interval.

2) There is a stronger correlation between species with low tissue ratios and low intervascular interval values than there is between species with high tissue ratios and high intervascular interval values.

3) The six species with the lowest intervascular interval values are associated with either dry-sun or dry-shade habitats.

4) Seven of the ten species with the highest intervascular interval values are associated with a moist-shade habitat.

From this study, then, the intervascular interval would seem to be correlated more directly with high transpiration rates, whether favored by the conditions of the habitat or the organization of the mesophyll. Even this possibility does not completely satisfy what was observed in the study. Delphinium tricorne, a sun species, had a low tissue ratio and a high intervascular interval value. In this species, the morphology of the leaf may influence the venation pattern more than either its mesophyll organization or its habitat. The leaves of Delphinium are deeply dissected into narrow pinnatifid segments. The smallest of these segments measured 2mm. in width. Larger veins were oriented parallel to the midvein of each segment. Widely spaced minor veins branched off of these veins and turned to eventually run parallel to them forming a venation pattern which could be described as "open-parallel." Due to this venation pattern there were few vein endings present in the cleared leaf samples (Plate 4, Fig. 2). This condition necessitated taking intervascular interval measurements between parallel minor veins. According to Milthorpe (1956), although venation and leaf form are not necessarily linked, linear leaves tend

to be single veined whereas ovate leaves show a multiveined pattern. Perhaps this would also apply to linear segments of leaves.

On the basis of what was observed in this study and what was read on similar studies, it is not possible to explain the unusually low intervascular interval value obtained for Aquilegia canadensis.

The bundle sheath, with and without extensions, has been shown to function in the distribution of materials throughout the leaf. Armacost (1944) demonstrated for fifty species an immediate absorption of dye from injected xylem elements by the bundle sheath and showed that sheath extensions, when present, quickly conducted the dye to the epidermal layers. Because of their limited conductive capacity, small veins can serve only limited areas of contiguous mesophyll (Wylie, 1938); the border parenchyma, or bundle sheath, greatly increases the surface of minor veins for mesophyll contacts (Plymale and Wylie, 1944).

Included in an anatomical study of the bundle sheath extension by Wylie (1952) were groups of woody and herbaceous plants from the northern temperate zone, southern and subtropical species, and south temperate species. Wylie found that bundle sheath extensions were lacking in many species but did occur in both woody and herbaceous plants of the temperate and tropical regions. They occurred in the leaves of northern deciduous trees to such an extent that they were

considered by Wylie to be a "peculiar specialization" of those leaves. He found the bundle sheath extension to be lacking in about half of the herbaceous plants of the north temperate zone in the study. In summarizing his results, Wylie reported the greatest frequency of the bundle sheath extension in the northern deciduous leaves which had the thinnest blades, the least amount of mechanical tissue, and the thinnest cuticle. In plants lacking the sheath extensions, the tissue thickness means were greater, the average increase greatest in the spongy mesophyll. In species lacking the bundle sheath extension, the mesophyll was interrupted only by larger, major veins. The minor veins were generally farther apart than in those leaves with the sheath extension.

In support of Wylie's findings, it can be noted by referring to Table I that the seven species which lack bundle sheath extensions, Delphinium tricorne, Caltha palustris, Ranunculus pensylvanicus, Hepatica americana, H. acutiloba, Paeonia albiflora, and Coptis groenlandica, are among the eleven species with the thickest leaf blades. With the exception of the two species of Hepatica, they are among the six species with the greatest total percentage of mesophyll (Table II). Caltha palustris, Ranunculus pensylvanicus, and Coptis groenlandica have the highest percentages of spongy mesophyll of the study group (Table VII). However, Paeonia albiflora and Delphinium

tricorne have relatively small amounts of spongy mesophyll. Considering the percentage of spongy mesophyll and the frequency of bundle sheath extensions, Anemone virginiana, A. cylindrica, Clematis verticillaris, and Thalictrum polygamum had the lowest relative amounts of spongy mesophyll of those species in which the bundle sheath extension was observed.

Concordant with Wylie's conclusions in reference to the correlation between the frequency of the bundle sheath extension and minor vein spacing, Aquilegia canadensis, Clematis verticillaris, and Thalictrum polygamum with a relatively high frequency of the sheath extension are three of the four species in this study having the smallest intervascular interval values. This correlation is not, however, without exception. Paeonia albiflora, in which there were no sheath extensions associated with minor veins observed, had the second smallest intervascular interval value in the study.

Haberlandt (1914) considered the "nerve parenchyma" (bundle sheath) and its extensions to be a physiologic link between the photosynthetic tissue and the efferent channels of the leaf. In order for the leaf to maintain an efficient rate of photosynthesis, the products of the synthesis must be quickly removed, according to Haberlandt, and it is therefore expected that plants should have evolved arrangements for the most direct removal of these products. Haberlandt observed that

when this principle of direct removal is ignored, the photosynthetic cells themselves serve for translocation. Haberlandt proposed three means by which this translocatory function could be performed by the photosynthetic tissue. These are as follows: flanged or arm palisade in which the flanges are oriented in relation to the flow of the translocatory stream; convergent palisade in which the "free" ends of the palisade cells converge toward the network of vascular bundles; the arrangement of the branches of the spongy mesophyll cells directed to the vascular reticulum. Ranunculus pensylvanicus, Caltha palustris, the two species of Hepatica and Paeonia albiflora, among the species in this study which lacked bundle sheath extensions, did have flanged palisade (Plate 2, Figs. 1 and 3). Convergent palisade was observed in Delphinium tricorne especially associated with some of the smaller veins. The vertical orientation of the spongy mesophyll cells in Coptis groenlandica (Plate 2, Fig. 9) was unique among the study species. Hepatica americana and H. acutiloba had the most compact spongy mesophyll observed in the study (Plate 3, Fig. 2).

In the Ranunculaceae the bundle sheath extension associated with minor venation is apparently not a constant anatomical feature. Insofar as it can be determined from the current study, leaves with the sheath extension are generally thinner with lower relative amounts of spongy mesophyll and a closer net of minor veins. In leaves lacking

bundle sheath extensions, the converse characteristics of internal organization were less apparent. Considering the number of species in this study and the number of observed exceptions to previously reported correlations, the relationship between bundle sheath extensions, blade thickness, mesophyll organization, and vein spacing does not seem sufficiently applicable to be regarded as a principle of foliar organization.

SUMMARY AND CONCLUSIONS

Extensive variation in internal structure was observed in the leaves of the twenty species of Ranunculaceae in this study.

Spongy mesophyll was the most variable blade tissue within the study group, accounting for from 62.3% of the dorsiventral depth in Caltha palustris to 34.3% in Thalictrum polygamum. In nineteen species the relative percentage of spongy mesophyll exceeded that of the palisade tissue, the exception being Thalictrum polygamum in which a greater relative percentage of palisade was observed in all leaf samples.

In an analysis of the mesophyll region as observed in thin and thick leaf samples of each species, three patterns of mesophyll organization and development were observed. Of the twenty species, 65% showed leaf thickness to be the result of spongy mesophyll development. In 25% of the species thickness was attributed to palisade elongation. Epidermal development accounted for leaf thickness in 10% of the species in the study. None of the mesophyll organization patterns could be correlated with habitat type.

The two species of the genus Hepatica had very similar internal foliar structure although their habitat types varied. The two species of Anemone in the study, occurring in similar habitats and having morphologically similar leaves, showed variation in internal

organization. The internal leaf structures of the species in the genus Ranunculus were distinct.

With the exception of a fibrous bundle sheath, no xeromorphy was observed in the evergreen bog species Coptis groenlandica.

As observed in this study, the spacing of minor veins was more closely associated with environment than with mesophyll organization per se; the smallest intervascular intervals were obtained from species from dry-sun or dry-shade habitats. It was noted that previous correlations between the relative volume of palisade tissue and minor vein spacing were based on leaf studies in which extensive palisade development is characteristic.

In species with finely dissected leaves, such as Delphinium tricorne in this study, it was suggested that leaf morphology may determine vascular patterns more than mesophyll organization or habitat.

The bundle sheath extension, when present, was apparently not constant along the entire course of a minor vein. Previously reported characteristic features of the internal anatomy of leaves having bundle sheath extensions seemed more consistent than features of leaves lacking the extensions.

Anatomical features which reportedly could be associated with translocatory functions were observed in species lacking the bundle sheath extension associated with minor venation.

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